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Draft NTP Brief on Soy Infant Formula: BSC Charge and Background on NTP Level of Concern Conclusions

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NIEHS/NTP/CERHR

May 10, 2010
NTP Board of Scientific Counselors

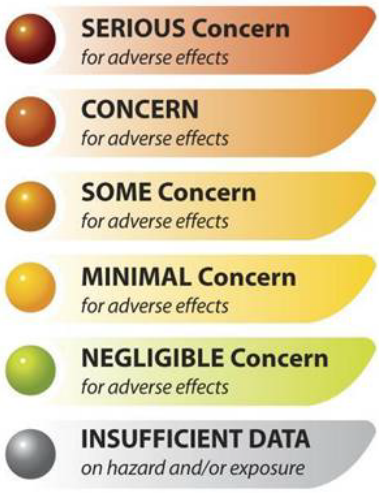
BSC Charge

To determine whether the scientific information cited in the draft NTP Brief on Soy Infant Formula is technically correct, clearly stated, and supports the NTP's conclusions regarding the potential for soy infant formula to cause adverse developmental effects.

Action: NTP BSC will vote on whether the science cited in the draft NTP Brief on Soy Infant Formula supports the conclusion of *minimal concern* for adverse effects on development in infants who consume soy infant formula.

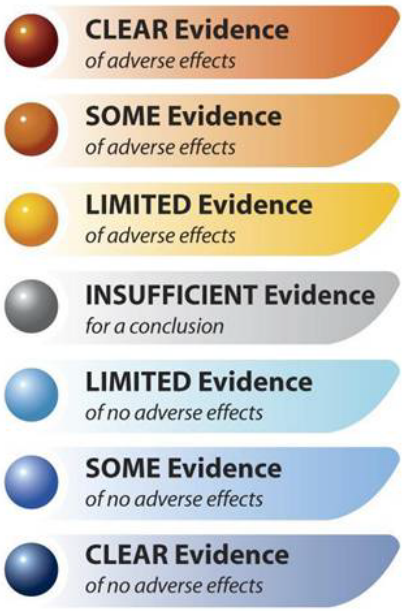
NTP Levels of Concern

- 5 category scale + a category for insufficient data
- No set scientific definitions for each category
- Integration of weight of evidence for adverse developmental/reproductive effects in humans and animals, extent of current human exposure, and other factors
- Evaluation can have multiple conclusions for different effects, life stages, or levels of exposure



Weight of Evidence for Adverse Effects

- 7-point hazard identification scale
- Human and animal data considered separately
- Conclusions reached on case by case basis



Example: Propylene Glycol (2004)

Weight of evidence for developmental and reproductive toxicity

Humans
(few studies)

Laboratory animals
(at high doses in multiple species)

- CLEAR Evidence of adverse effects
- SOME Evidence of adverse effects
- LIMITED Evidence of adverse effects
- INSUFFICIENT Evidence for a conclusion
- LIMITED Evidence of no adverse effects
- SOME Evidence of no adverse effects
- CLEAR Evidence of no adverse effects

+

Extent of human exposure and other factors

Human exposure

- Occupational exposure not considered excessive
- Short half-life in humans

Other factors

- Saturation of metabolic step at lower doses in humans compared to animals ("protective")

=

Level of concern

- SERIOUS Concern for adverse effects
- CONCERN for adverse effects
- SOME Concern for adverse effects
- MINIMAL Concern for adverse effects
- NEGLIGIBLE Concern for adverse effects
- INSUFFICIENT DATA on hazard and/or exposure

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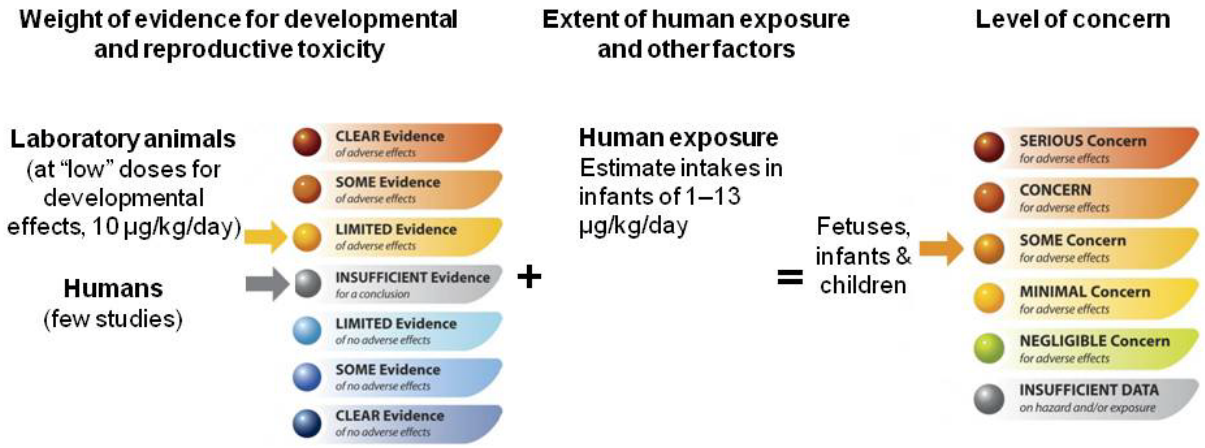
Example: Bisphenol A (2008)



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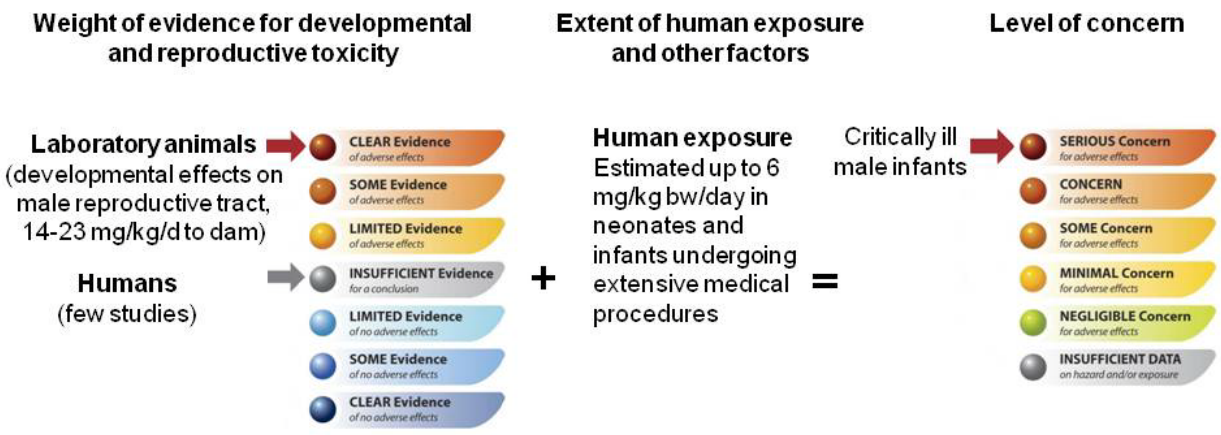
Example: Di(2-Ethylhexyl) Phthalate (DEHP, 2006)



Questions on Charge?

To determine whether the scientific information cited in the draft NTP Brief on Soy Infant Formula is technically correct, clearly stated, and supports the NTP's conclusions regarding the potential for soy infant formula to cause adverse developmental effects.

Action: NTP BSC will vote on whether the science cited in the draft NTP Brief on Soy Infant Formula supports the conclusion of *minimal concern* for adverse effects on development in infants who consume soy infant formula.

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Draft NTP Brief on Soy Infant Formula

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Outline of Presentation

- Background and scientific development of draft NTP Brief on Soy Infant Formula
- Usage and exposure to isoflavones in infants fed soy formula

BSC Discussion

- Weight of evidence conclusions for adverse effects on development based on human studies

BSC Discussion

- Weight of evidence conclusions for adverse effects on development based on laboratory animal studies

BSC Discussion

- Draft NTP level of concern conclusion for soy infant formula

BSC Discussion and Vote

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Background and Scientific Development of Draft NTP Brief on Soy Infant Formula

Soy Infant Formula

- Used since 1950s to replace or supplement use of breastmilk or cow milk-based formula
- Contains soy protein isolate at 14-16% by weight
  - Soy isoflavones with estrogenic activity ("phytoestrogens")
    - Genistein > daidzein > glycitein (relative abundance in soy infant formula and relative estrogenic activity)
  - Less variability in isoflavone content than other soy products
    - 20.9 to 47 mg/L total isoflavones for US samples
      - genistein is 58 to 66% of isoflavone content

Basis for NTP Evaluation

- Availability of studies in humans and laboratory animals relevant for an assessment of developmental toxicity
- Availability of information on isoflavone exposure in infants fed soy formula
- Public concern
- Update of 2006 NTP evaluation that was not completed

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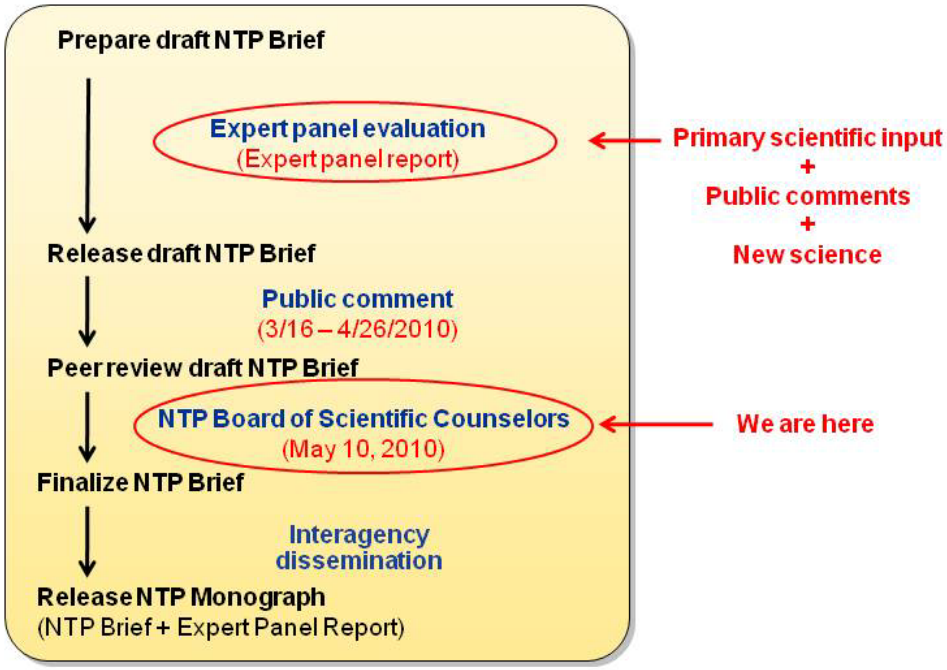
Scientific Development of Draft NTP Brief for Soy Infant Formula



Expert Panel Evaluation

- Expert panel expressed "minimal concern" for adverse developmental effects in infants fed soy formula (public meeting held December 16-18, 2009)
  - Meeting deliberations based on draft expert panel report released for public comment in October 2009
  - Final expert panel report released January 15, 2009 for public comment
- Expert panel conclusions based on:
  - Critical assessment of relevant human and animal studies of developmental toxicity
    - Classified studies as "no," "limited," or "high" utility
    - Developed weight of evidence conclusions for developmental toxicity based on "limited" or "high" utility studies
  - Extent of isoflavone exposure in infants fed soy formula

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Usage and Exposure

Usage of Soy Infant Formula

- ~12% of US infant formula market based on 2009 dollar sales
  - Decreased from 22.5% in 1999
- Unknown how many infants exclusively fed soy formula
  - ~60-70% infants fed some type of formula in first 10 months of life
  - Changes in feeding method are common

American Academy of Pediatrics 2008 Policy Statement on Soy Infant Formula

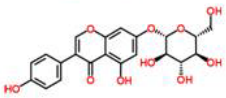
- Can provide nutrition for normal growth and development in term infants, but limited clinical indications for use
  - Galactosemia or primary lactase deficiency (rare)
  - Vegetarian diet
- Specific conclusions and recommendations:
  - No proven value for colic or prevention of atopic disease
  - Lactose free or reduced lactose-containing cow milk formula for lactose intolerance
  - Extensively hydrolyzed protein formula for infants with cow milk protein allergy
  - Hydrolyzed protein or synthetic amino acid formula for cow milk protein-induced enteropathy or enterocolitis
  - Not recommended for preterm infants (risk of osteopenia)

Bhatia (2008) Pediatrics 121(5): 1062-1068

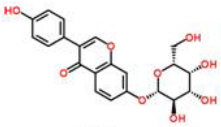
Isoflavones Associated with Soy Infant Formula

Sugar-bound isoflavones found in soy infant formula
(not biologically active)

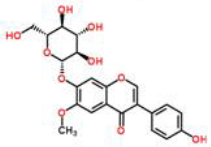
Genistin
MW: 423.37
58-66% of isoflavone content



Daidzin
MW: 416.37
29-34% of isoflavone content



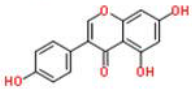
Glycitin
MW: 446.41
5-8% of isoflavone content



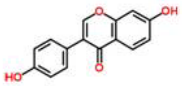
Glucoside often expressed in "aglycone equivalents" based on molecular weight e.g., 1µM genistin (MW 432.38) = 0.63 µM genistein (MW 270.2)

Aglycone isoflavones found in blood and tissues
(biologically active)

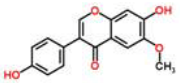
Genistein
MW: 270.24



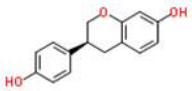
Daidzein
MW: 254.24



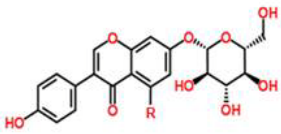
Glycitein
MW: 284.26



Equol
MW: 242.27

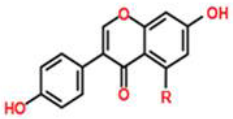


Biotransformation of Genistein and Daidzein



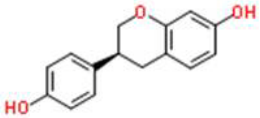
GLUCOSIDE FORMS
genistin: R = OH/daidzin: R = H
(predominant forms in soy infant formula)

↓ microbial and intestinal β-glucosidases



AGLYCONE FORMS ("UNCONJUGATED")
genistein: R = OH/daidzein: R = H
(biologically active form; 1-3% in blood)

→→ Metabolism of daidzein



Equol
(produced in ~30–50% humans)
mostly circulates in conjugated form

↓ Intestinal and hepatic UGTs and SULTs

GLUCURONIDE AND SULFATE CONJUGATES
genistein/daidzein 7- and 4'-glucoronides
genistein/daidzein 7'-sulfate

In Vitro Measures of Relative Estrogenicity

- Genistein ≈ equol > daidzein > glycitein

	Relative estrogenic potencies			Relative binding affinity (%)		
Compound	ER binding	Yeast transactivation	E-screen	ERα	ERβ	β/α
E_2	++++	++++	++++	100	100	1
Genistein	+++	++++	+++	2.07	14.8	7.1
Equol	++	+++	++++	1.70	4.45	2.6
Daidzein	++	++	++	0.55	0.46	0.8
Glycitein	++	not determined	++	0.32	0.44	1.4

Choi (2008) Planta Med 74(1): 25-32

Few *In Vivo* Studies Directly Comparing Estrogenic Activities of Isoflavones

- Some suggestion that estrogenicity of equol less than predicted based on *in vitro* studies
  - "Classic" estrogenic response, athymic mouse MCF-7 xenograph mammary gland tumor model
- Greater percent of equol conjugated compared to genistein
  - 9% unconjugated genistein versus 1% unconjugated equol in Balb/c mice following ingestion of soy flour diet [Allred (2005) J Agric Food Chem 53(22): 8542-8550]
  - 1-5% unconjugated genistein (after genistin treatment) versus < 0.3% unconjugated equol in infant and adult rhesus monkeys (after daidzin treatment)(Dan Doerge, NCTR/FDA, personal communication)

Estimated Daily Intake of Total Isoflavones and Genistein

Population	Daily Intake (mg/kg bw/day)	
	Total Isoflavone	Genistein
Infants		
Soy infant formula	2.3 – 9.3	1.3 – 6.2
Cow milk formula	0.0002 - 0.0158	
Breastmilk	0.0002 - 0.0063	
Adults		
US, omnivore	0.0097 – 0.096	0.005 – 0.056
US, vegetarian	0.21	0.14
UK, vegan	1.07	–
Japanese, traditional diet	0.67	0.077 – 0.43

Data Available on Blood Levels of Isoflavones in Infants

- Largest study is a relatively new publication [Cao (2009) J Expo Sci Environ Epidemiol 19(2): 223-234]
  - Blood, urine, saliva levels of total genistein, daidzein, and equol in infants fed soy formula, cow milk formula, or breastmilk
    - Infants 0 to 12 months of age
    - Chemical analysis done at CDC and NCTR
  - Sample sizes for whole blood ranged from 20-30 infants
    - 27 soy formula-fed infants
  - Too little blood volume collected for measurement of unconjugated (heel or toe prick)
  - Blood samples collected 30 to 120 minutes after morning feeding
- Reported blood levels consistent with previous data based on 7 male infants [Setchell (1997) Lancet 350(9070): 23-27]

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Blood-Based Levels of Genistein and Daidzein in Infants and Adult Populations

Population, diet	Average Total Isoflavone Concentration, ng/ml		Reference
	Genistein	Daidzein	
Infants			
Soy infant formula	684–757	256 – 295	Setchell 1997; Cao 2009
	1455, 75th percentile	519, 75th percentile	Cao 2009
	2764, 95th percentile		Cao 2009, personal communication
Cow milk formula	3.2 –14.2	2.1 – 5.5	Setchell 1997; Cao 2009
Breastfed	2.8 –10.8	1.5 –5.3	Setchell 1997; Cao 2009
Adults			
US adults, omnivores	4.7 (<LOD – 203, range)	3.9 (<LOD – 162, range)	Valentin-Blasini 2003
Japanese men, traditional diet	105.2 (24 – 325, range)	71.3 (14.8 – 234.9, range)	Adlercreutz 1994
UK adults, vegans/vegetarians	40	20	Peeters 2007

Valentin-Blasini (2003) J Expo Anal Environ Epidemiol 13(4): 276-282; Adlercreutz (1994) Cancer Detect Prev 18(4): 259-271; Peeters (2007) J Nutr 137(5): 1294-1300

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Questions and Discussion on Usage and Exposure

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Weight of Evidence Conclusions for Developmental Toxicity (Human Studies)

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The Weight of Evidence that Soy Infant Formula Causes Adverse Developmental Effects in Humans?

Developmental toxicity[1] → **INSUFFICIENT Evidence** *for a conclusion*

Growth in healthy full-term infants
Based on overall pattern of results from "limited utility" studies and a large number of "no utility" studies reporting similar growth profiles in infants fed soy formula compared to other feeding methods →

- **CLEAR Evidence** *of adverse effects*
- **SOME Evidence** *of adverse effects*
- **LIMITED Evidence** *of adverse effects*
- **LIMITED Evidence** *of no adverse effects*
- **SOME Evidence** *of no adverse effects*
- **CLEAR Evidence** *of no adverse effects*

[1] Based on consideration of the following endpoints: bone mineral density, allergy/immunology, thyroid function, reproductive endpoints, cholesterol, diabetes mellitus, and cognitive function

Human Studies

- Evaluation focused on studies of infants fed soy formula
  - No studies of genistein or other individual isoflavones
- Most studies considered "no" utility
  - 28 of ~80 studies considered of "limited" utility
  - None were of "high" utility
- Common limitations included:
    - Inadequate sample size
    - Changes in feeding methods
    - Short-duration of follow-up
    - No validation of exposure to soy formula
    - Inadequate consideration of potential confounding variables

"Insufficient" Evidence to Reach a Conclusion on Reproductive Endpoints

- 3 studies of "limited" utility in the final expert panel report
- 1 new study published since the expert panel meeting

"Insufficient" Evidence to Reach a Conclusion on Reproductive Endpoints: 3 Studies of "Limited" Utility in Expert Panel Evaluation

1. Strom (2001):Retrospective cohort study of men and women aged 20-34 years who participated in non-randomized feeding study as infants
   - Soy formula (120 men, 128 women) and cow milk formula (295 men, 268 women)
   - Telephone interviews/self-reported pubertal maturation, menstrual and reproductive history, height and usual weight, current health
   - Duration of menstrual bleeding was 0.37 days longer and severe menstrual discomfort was more common in women fed with soy formula than with cow-milk formula
     - Not statistically significant after adjustment for multiple comparisons
   - Main reasons considered "limited" utility by expert panel: non-random feeding assignment, short-term exposure (0-16 weeks), statistically underpowered for some endpoints (e.g., cancer, reproductive/hormonal disorders), measures of infertility considered weak (74/128 fed soy formula tried to become pregnant, "attempted pregnancy without success")

Strom (2001) Jama 286(7): 807-814

"Insufficient" Evidence to Reach a Conclusion on Reproductive Endpoints: 3 Studies of "Limited" Utility in Expert Panel Evaluation

2. Freni-Titulaer (1986): Case-control study of girls with premature thelarche (n=130) and age-matched controls; retrospective questioning of parents about feeding method
   - No overall significant association between premature thelarche and soy infant formula intake
   - Restriction of multivariate analysis to subjects with thelarche before age 2 years showed significant association
     - NTP did not consider sufficient evidence of an association when studies of "no" utility related to breast development were considered
   - Other significant factors included maternal ovarian cysts and consumption of chicken; consumption of corn was protective

Freni-Titulaer (1986) Am J Dis Child 140(12): 1263-1267

"Insufficient" Evidence to Reach a Conclusion on Reproductive Endpoints: 3 Studies of "Limited" Utility in Expert Panel Evaluation

3. Boucher (2008): Adults with breast cancer (n=372) and without breast cancer (n=356) who had been fed soy formula, cow-milk formula, or breastmilk
   - A reduced, but non-significant, association between soy formula intake and breast cancer in adults

Boucher (2008) Epidemiology 19(1): 165-166

"Insufficient" Evidence to Reach a Conclusion on Reproductive Endpoints: New Study by D'Alosio (2010)

- Reported association between soy formula use during infancy and early uterine fibroid diagnosis in adulthood
- 19,972 non-Hispanic white women ages 35 to 59 at enrollment in the NIEHS Sister Study.
  - Relative risk for "ever" versus "none" on soy infant formula use = 1.25, 95% confidence interval of 0.97 – 1.61)
  - Relative risk for "yes" versus "no" on soy infant formula use ≤ 2 months of age = 1.25; 95% CI: 0.90, 1.73)

D'Aloisio (2010) Environmental Health Perspect 18(3): 375-381

"Insufficient" Evidence to Reach a Conclusion on Reproductive Endpoints: New Study by D'Alosio (2010)

- Limitations on assessment of soy infant formula use
  - Use of family history questionnaire; dichotomous responses for soy infant formula use
- Confidence intervals in D'Aloisio (2010) overlap with 1
- Use of soy infant formula also associated with both higher odds of very early menarche (<11 yrs) and late menarche (abstract; D'Aloisio 2009)
- No increase in uterine fibroids or endometriosis in Strom 2001 study (Goldman 2001)

	Soy formula (n=128)	Cow milk formula (n=268)
Uterine fibroids	0 (0%)	6 (2.2%)
Endometriosis	0 (0%)	6 (2.2%)

D'Aloisio (2010) Environmental Health Perspect 18(3): 375-381; D'Aloisio (2009) AEP 19(9): 651-680; Goldman (2001) Jama 286(19): 2402-2403

Monitoring Thyroid Function in Infants with Congenital Hypothyroidism Fed Soy Formula Identified as Research Need

- Based on 4 case reports/series with sample sizes of 1 to 8
  - "Insufficient evidence" for a conclusion for adverse developmental effects on thyroid
- Infants with congenital hypothyroidism on a soy formula diet may require higher doses of L-thyroxin for treatment
  - Effect attributed to fecal wastage with decreased enterohepatic circulation

Some Data Gaps May be Addressed by Ongoing Prospective Studies

- Arkansas Children's Nutrition Center
- Infant Feeding and Early Development (IFED)

Arkansas Children's Nutrition Center Prospective Cohort Study

- Infants fed soy formula, cow milk formula, and breastmilk
  - Growth, development, body composition, endocrine status, metabolism, organ development, cognitive function, language acquisition, and psychological development
  - Assessment at 3, 6, 9, 12, and 18 months and at 2, 3, 4, 5, and 6 years
- An interim report was published by Gilchrest (2009)
  - 4 month old infants assessed for growth and reproductive organ development
    - 18 – 20 infants per sex in each group
  - Considered of "no utility" for evaluation because:
    - Considerable cross-feeding (only 23% of infants in soy formula group entirely fed soy infant formula)
    - Duration of use ranged from 2 to 4 months

Infant Feeding and Early Development (IFED) Prospective Cohort Study

- Funded by NIEHS and conducted at Children's Hospital of Philadelphia
  - Recruitment expected to begin in spring 2010
- Infants fed soy formula, cow milk formula, and breastmilk
  - Stricter criteria for feeding regimen eligibility compared to Arkansas Children's Nutrition Center cohort study
- Infant phase to enroll up to 450 mothers/infant pairs
  - Changes in estrogen responsive tissues; repeated visits during infancy ultrasound (uterus, ovaries, testes, breast, thyroid) at half the visits
  - Pilot study to characterize estrogen responsive endpoints in infants considered by expert panel (Bernbaum 2008)
    - "No" utility because it was a small sample size pilot study
- Toddler phase to enroll up to 1500 toddlers
  - Language acquisition/hearing assessment, toy preference, physical exam, bone density

Questions & Discussion on Weight of Evidence Conclusions Based on Human Studies

Developmental toxicity[1] → **INSUFFICIENT Evidence** *for a conclusion*

Growth in healthy full-term infants
Based on overall pattern of results from "limited utility" studies and a large number of "no utility" studies reporting similar growth profiles → **SOME Evidence** *of no adverse effects*

- **CLEAR Evidence** *of adverse effects*
- **SOME Evidence** *of adverse effects*
- **LIMITED Evidence** *of adverse effects*
- **INSUFFICIENT Evidence** *for a conclusion*
- **LIMITED Evidence** *of no adverse effects*
- **SOME Evidence** *of no adverse effects*
- **CLEAR Evidence** *of no adverse effects*

[1] Based on consideration of the following endpoints: bone mineral density, allergy/immunology, thyroid function, reproductive endpoints, cholesterol, diabetes mellitus, and cognitive function

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Weight of Evidence Conclusions for Developmental Toxicity (Laboratory Animal Studies)

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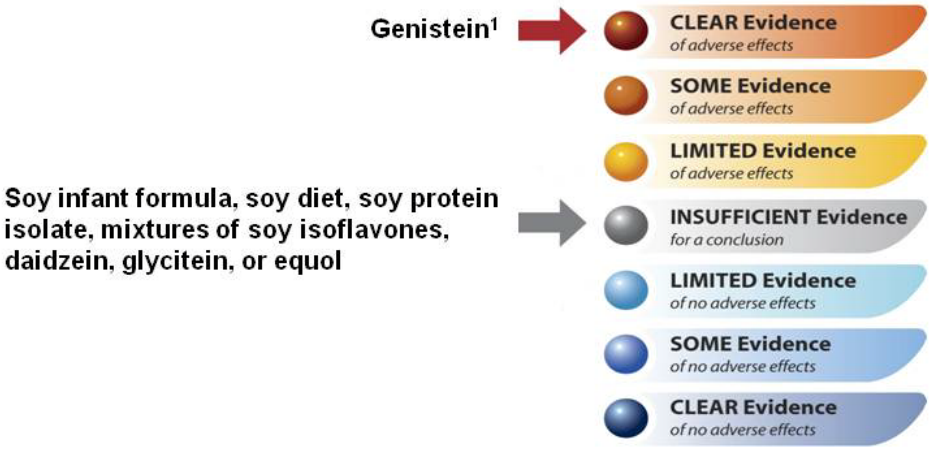
Weight of Evidence that Soy Infant Formula, Other Soy Products, or Individual Isoflavones Cause Adverse Developmental Effects in Laboratory Animals



[1]Manifested as: decreased age at vaginal opening; abnormal estrous cyclicity; decreased fertility, implants, and litter size; and histopathology of the female reproductive tract.

Laboratory Animal Studies

- Very few studies of soy infant formula
- Most studies of genistein, soy diet, soy protein isolate, or mixtures of soy isoflavones
- Life stage at exposure was a major factor considered by expert panel in determining study utility
  - Studies with treatment outside the window of lactation (PND1-21) were "limited" utility at most

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Soy Infant Formula Studies in Laboratory Animals

"Insufficient" Evidence to Reach a Conclusion on Potential Adverse Developmental Effects Based on Laboratory Animal Studies with Soy Infant Formula

- 3 studies considered
  - Ashby (2000) rat study of "no utility"
    - Treatment began after period of lactation
  - Two marmoset monkey studies of "limited" utility based on same group of animals assessed at different ages (Sharpe 2002; Tan 2006)

Sharpe (2002) Hum Reprod 17(7): 1692-1703
Tan (2006) Hum Reprod 21(4): 896-904.

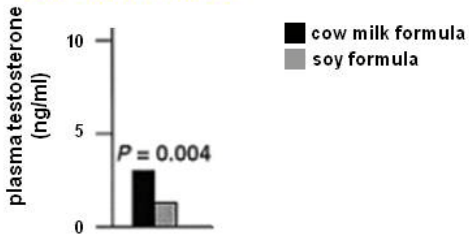
Male Marmosets Fed Soy Formula as Infants

- Co-twin study with each twin set fed cow milk-based or soy formula
  - Estimated isoflavone intake less than intake in human infants exclusively fed soy formula
    - Infants hand fed formula ~ 8 hours each day (2 hours on weekends) from 4–5 to 35–45 days of age
    - No direct measurements of isoflavone in formula or animals
  - Animals assessed at 35–45 days of age (Sharpe 2002) or as adults at 120–138 weeks of age (Tan 2006)
    - 13 co-twins + 2 non-twin males (13–15 per formula group) assessed at 35–45 days of age
    - 7 co-twins (7 per formula group) assessed at 120–138 weeks of age (~30–138 months)

Plasma Testosterone in Male Marmosets Fed Soy Formula as Infants

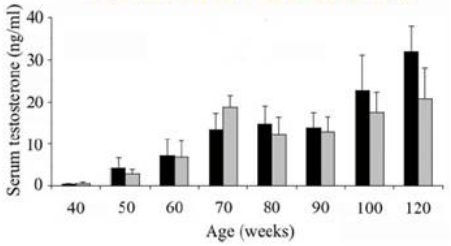
- Reduced plasma testosterone levels at end of treatment but not in adulthood

35–45 days of age



Group (n)	Number of animals at 35-45 days of age	
	"low" testosterone <0.5 ng/ml	testosterone >0.5 ng/ml
Historical controls (22)	2	20
Cow milk formula (15)	1	14
Soy formula (15)	12***	3

Up to 120 –138 weeks of age

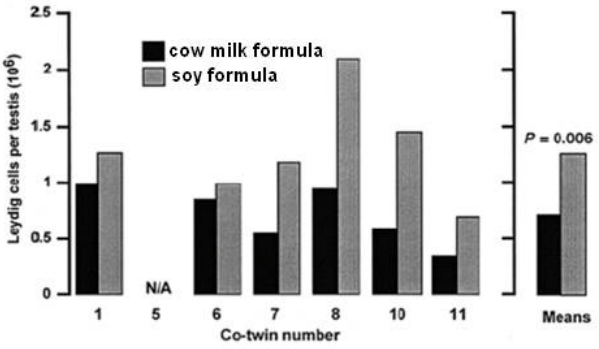


No difference in adult testosterone levels or onset of puberty

Sharpe (2002) Hum Reprod 17(7): 1692-1703
Tan (2006) Hum Reprod 21(4): 896-904

Testicular Effects in Male Marmosets Fed Soy Formula as Infants at 35–45 Days of Age

- Increased number of Leydig cells per testis
- No effects on testicular size or number of Sertoli or germ cells per testis

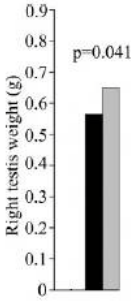


Sharpe (2002) Hum Reprod 17(7): 1692-1703

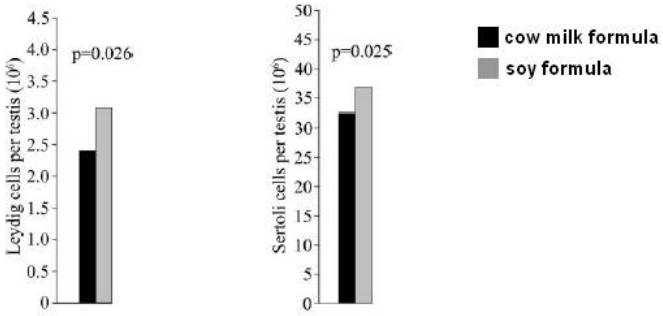
Testicular Effects in Male Marmosets Fed Soy Formula as Infants at 120 –138 weeks of age

- Increased testis size and altered cell composition, possible "compensated Leydig cell failure"
- No gross adverse reproductive effects, i.e., fertility

Testis weight



Leydig and Sertoli cell composition



Tan (2006) Hum Reprod 21(4): 896-904

Interpretation and Limitations of the Marmoset Soy Infant Formula Studies

- Functional adversity of testicular effects unclear
- Infants fed soy formula and nursed by mother
  - Estimated intake lower than human infants
- Small sample size to assess certain endpoints, i.e., fertility

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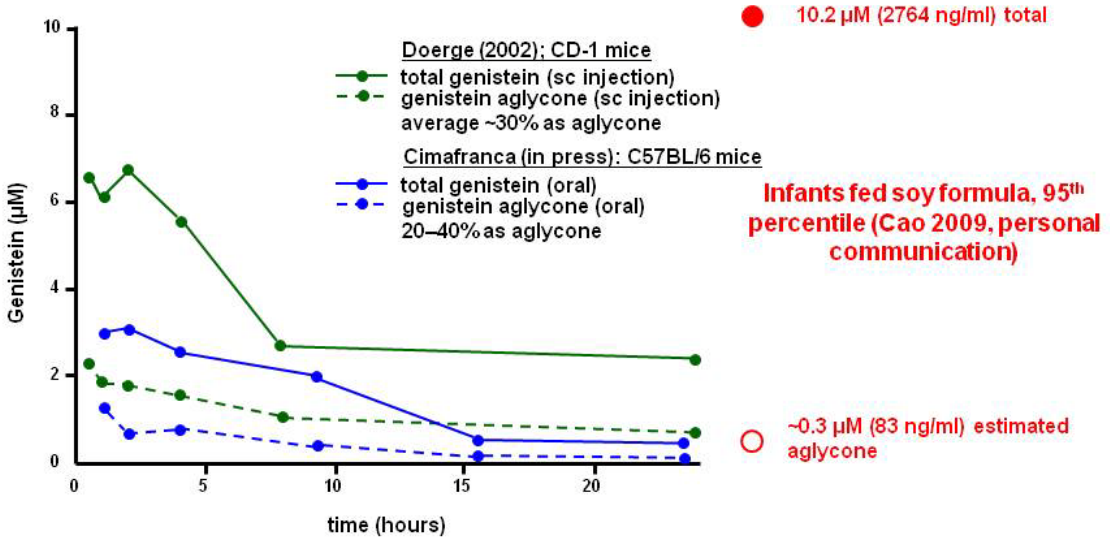
Genistein/Genistin Studies in Laboratory Animals

"Clear" Evidence of Adverse Effects of Genistein & Genistin on Development

- Mouse studies with treatment only during lactation on PND 1–5
  - Genistein (sc injection)
    - SC injection results in greater portion unconjugated (biologically active) in adult rodents
      - More similar conjugation patterns between oral and sc injection in neonatal mice compared to adult mice or adult rats
    - Ability to interpret sc injections studies increased because blood levels have been characterized
  - Genistein (oral)
  - Genistin (oral)
- Rat NTP multigenerational study with treatment during gestation, lactation, and post-weaning
- Blood levels of genistein (total and aglycone) characterized in these studies

Genistein Blood Levels in Neonatal Mice Treated on PND 1–5 with 50 mg/kg/d Genistein by SC Injection (CD-1 Mice) or Orally (C57BL/6)



Doerge (2002) Cancer Lett 184(1): 21-27; Cimafranca (in press) Biol Reprod. Online March 31, 2010)

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Treatment During Lactation Only
Genistein (SC Injection, PND 1–5)

Reduced Fertility in CD-1 Mice Following Treatment with Genistein During PND1–5 (SC Injection)

Age (months)	Dose (mg/kg/d)	# Mice with litters/plug positive (%)	Average # live pups
2	control	6/6 (100)	15.2
	0.5	6/6 (100)	13.2
	5	6/8 (75)	11.5
	50	0/16 (0)	0
6	control	7/7 (100)	13.7
	0.5	3/5 (60)	9.3
	5	2/5 (40)	8.5
	50	--	--

From Jefferson (2005) Biol Reprod 73: 798-806

- Lower number of live pups at 5 mg/kg/d when data from all ages combined
- No live pups in 50 mg/kg/day treatment group also reported in Padilla-Banks (2006) Endocrinology 147(10): 4871-4882

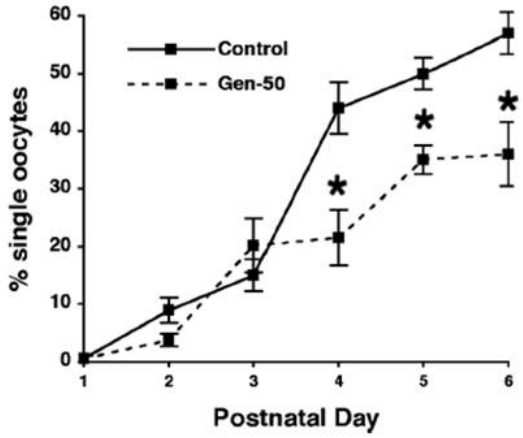
Multioocyte Follicles in CD-1 Mice Following Treatment with Genistein During PND1–5 (SC Injection)

Number of CD-1 and C57BL/6 Mice with at least 1 multioocyte follicle on PND 19

	Genistein (mg/kg bw/day)			
Strain	**Vehicle**	**0.5**	**5**	**50**
CD-1	0/8	1/8	2/8	6/8
C57BL/6	1/11	1/11	9/11	11/11

Jefferson (2002) Biol Reprod 67(4): 1285-1296.

CD-1 mice treated with 50 mg/kg



Jefferson (2006) Biol Reprod 74: 161-168.

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Treatment During Lactation Only
Genistein (Oral, PND 1–5)

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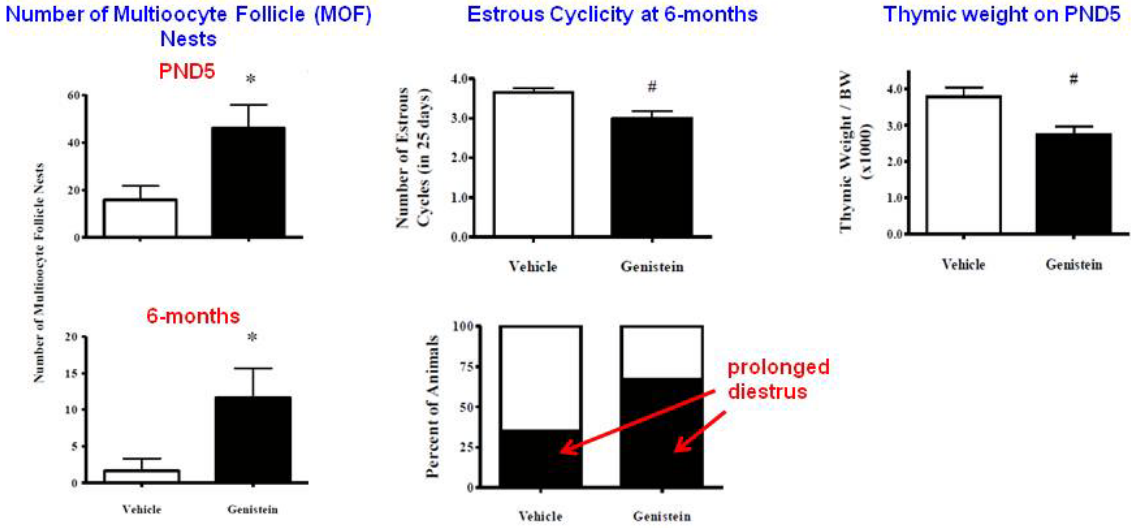
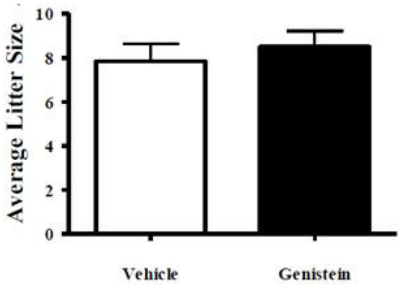
Effects on Ovary, Estrous Cycle, and Thymus in C57BL/6 Mice Treated with 50 mg/kg/d Genistein During PND1–5 (Oral)

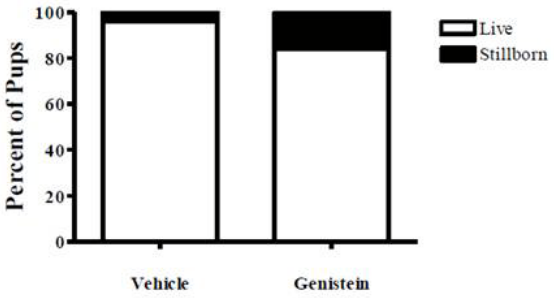


Cimafranca (in press) Biol Reprod. Online March 31, 2010)

No Effects on Fertility in C57BL/6 Mice Treated with 50 mg/kg/d Genistein During PND1–5 (Oral)

- Also no effect on age at vaginal opening





Cimafranca (in press) Biol Reprod. Online March 31, 2010)

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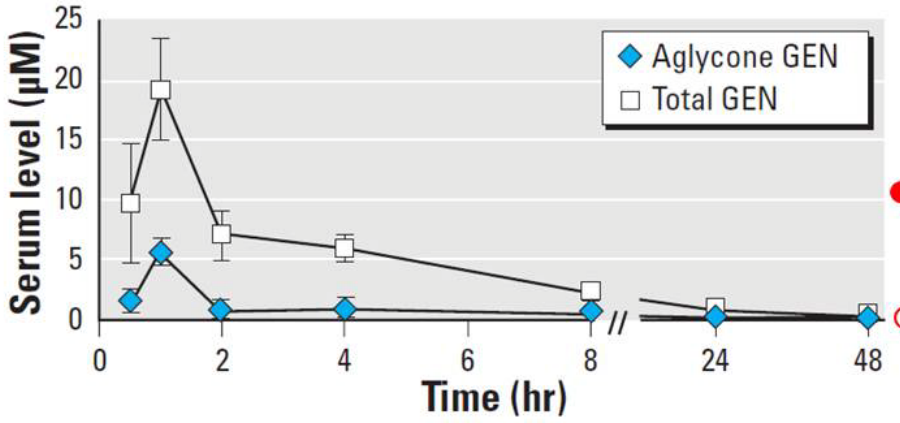
Treatment During Lactation Only
Genistin (Oral, PND 1–5)

Genistein Blood Levels in Neonatal CD-1 Mice Treated with 37.5 mg/kg/d Genistin (Oral, Aglycone Equivalents) and Infants Fed Soy Formula



Infants fed soy formula, 95th percentile (Cao 2009, personal communication)

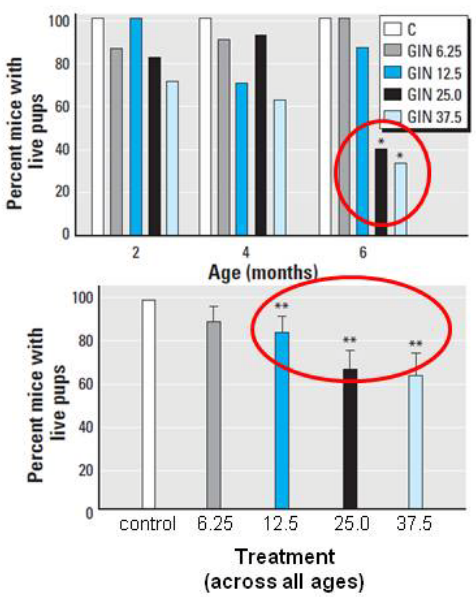
● 10.2 μM (2764 ng/ml) total

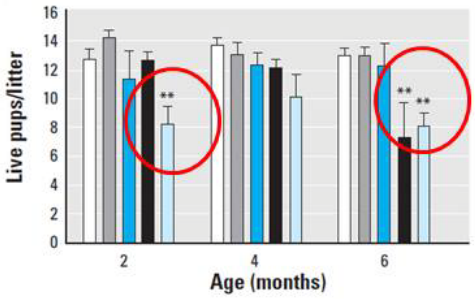
○ ~0.3 μM (83 ng/ml) estimated aglycone

Jefferson (2009) EHP 117(12): 1883-1889.

Reduced Fertility in CD-1 Mice Following Treatment with Genistin During PND1–5 (Oral)

- GIN = genistin, dose level expressed in aglycone equivalents

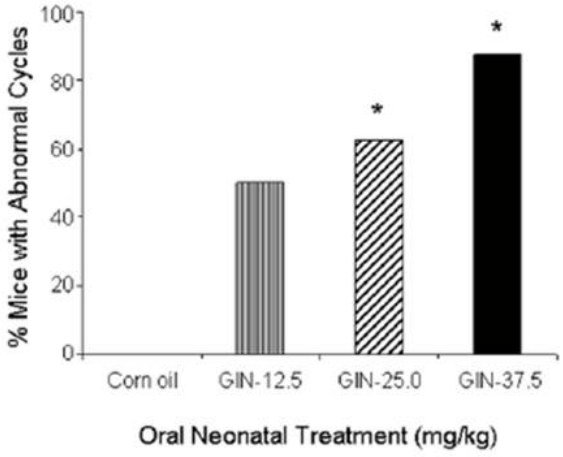




Jefferson (2009) EHP 117(12): 1883-1889.

Abnormal Estrous Cyclicity in CD-1 Mice Following Treatment with Genistin During PND1–5 (Oral)

- Estrous cyclicity monitored at 2 months of age for 2 weeks
- Effect attributed to prolonged time in estrus

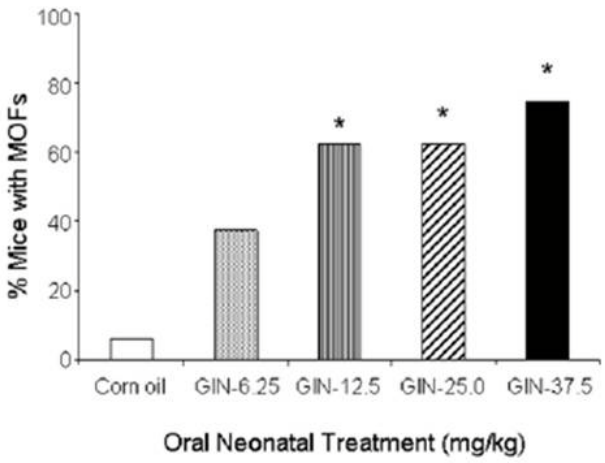


GIN = genistin, dose level expressed in aglycone equivalents

Jefferson (2009) EHP 117(12): 1883-1889.

Increased Multioocyte Follicles in CD-1 Mice Following Treatment with Genistin During PND1–5 (Oral)

- Presence of multioocyte follicles evaluated on PND19



GIN = genistin, dose level expressed in aglycone equivalents

Jefferson (2009) EHP 117(12): 1883-1889.

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NTP Multigeneration Rat Study

NTP Multigeneration Sprague-Dawley Rat Study (TR539)

- Five generation study with rats fed 0, 5, 100, 500 ppm genistein
  - Average dose of ~35 mg/kg/d in males to 51 mg/kg/d in females
- Considered "limited" utility by the expert panel
  - Treatment period included gestation and post-lactation
- Major findings at 500 ppm
  - Up to 30% decrease in litter size
  - ↓ Pre- and postweaning body weight
  - Up to ~3 day acceleration in vaginal opening
  - Altered estrous cyclicity
  - Male mammary gland hyperplasia
    - Also reported at 100 ppm in TR539 and 25 ppm in a dose range finding study

Comparison of Genistein Blood Levels in Rats at 500 ppm Genistein Infants Fed Soy Formula

Blood genistein		
Total genistein, ng/ml	Aglycone, ng/ml (%)	
2764	27.6 – 82.9 (1-3)*	Infants fed soy infant formula, 95th percentile (personal communication, Cao 2009)
2145 (female) 1620 (male)	21.5– 107.3 (female) 16.2 – 81 (male) (1-5%)	Rats on PND 140 (Chang 2000)
1455	14.6 – 43.7 (1-3)*	Infants fed soy infant formula, 75th percentile (Cao 2009)
891	8.9 – 26.7 (1-3)*	Infants fed soy infant formula, median (Cao 2009)
505 (female) 564 (male)	5.1 – 25.3 (female) 5.6 – 28.2 (male) (1-5%)	Rats on PND21 (Chang 2000)

*Estimated from adult percentage aglycone of 1-3%

Limitations of the Animal Literature for Reaching Conclusions on Soy Infant Formula

- Strongest evidence for adverse effects based on genistein-only studies
  - Studies of soy infant formula provided "insufficient" evidence to reach a conclusion
    - Too few studies and uncertainty on functional impact of effects observed in marmosets
- Studies of soy diet, soy protein isolate, or mixtures of soy isoflavones provided "insufficient" evidence to reach a conclusion
  - Findings often inconsistent
  - Difficult to reconcile due to variations in experimental design, administered form of soy product, dose levels tested, and treatment protocols

Limitations of the Animal Literature for Reaching Conclusions on Soy Infant Formula

- Possible impacts from other ingredients found in soy infant formula
  - Non-isoflavone ingredients
    - Corn syrup, vegetable oils, sugar, vitamins, minerals and other nutrients
    - Contaminants include:
      - Phytates (bind minerals and niacin)
      - Protease inhibitors (have antitrypsin, antichymotrypsin, and antielastin properties)
      - Minerals added to compensate for phytate binding and heated to inactivate protease inhibitors
  - Interactions between genistein and other isoflavones

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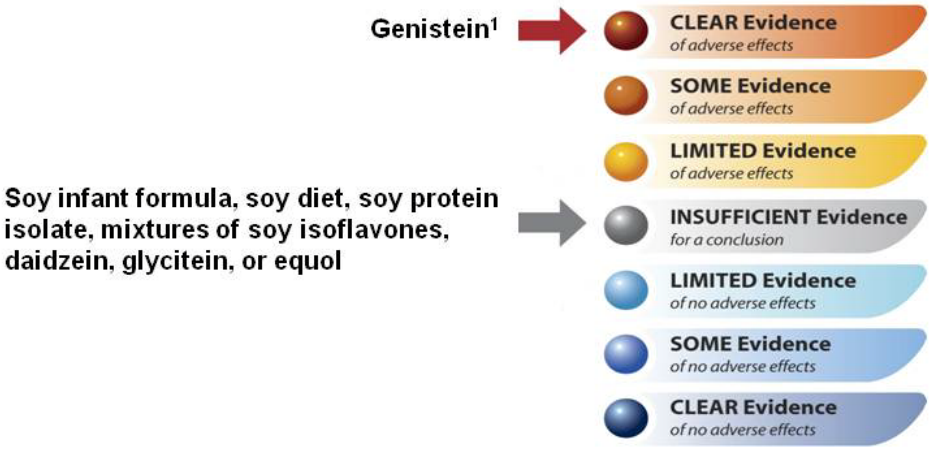
Questions & Discussion on Weight of Evidence Conclusions Based on Laboratory Animal Studies



[1]Manifested as: decreased age at vaginal opening; abnormal estrous cyclicity; decreased fertility, implants, and litter size; and histopathology of the female reproductive tract.

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Draft NTP Level of Concern Conclusion

Can Soy Infant Formula or its Isoflavone Contents Adversely Affect Human Development?

- Possibly*, based on:
  - Clear evidence of adverse effects of genistein in laboratory animals
  - Similarity in blood levels of genistein in infants fed soy formula to laboratory animals treated with dose levels of genistein or genistin that caused adverse effects

*Answers to this question may be: *Yes, Probably, Possibly, Probably Not, No, or Unknown*

Should Feeding Infants Soy Infant Formula Cause Concern?

The NTP concurs with the conclusion of the CERHR Expert Panel on Soy Infant Formula that there is *minimal concern* for adverse effects on development in infants who consume soy infant formula.

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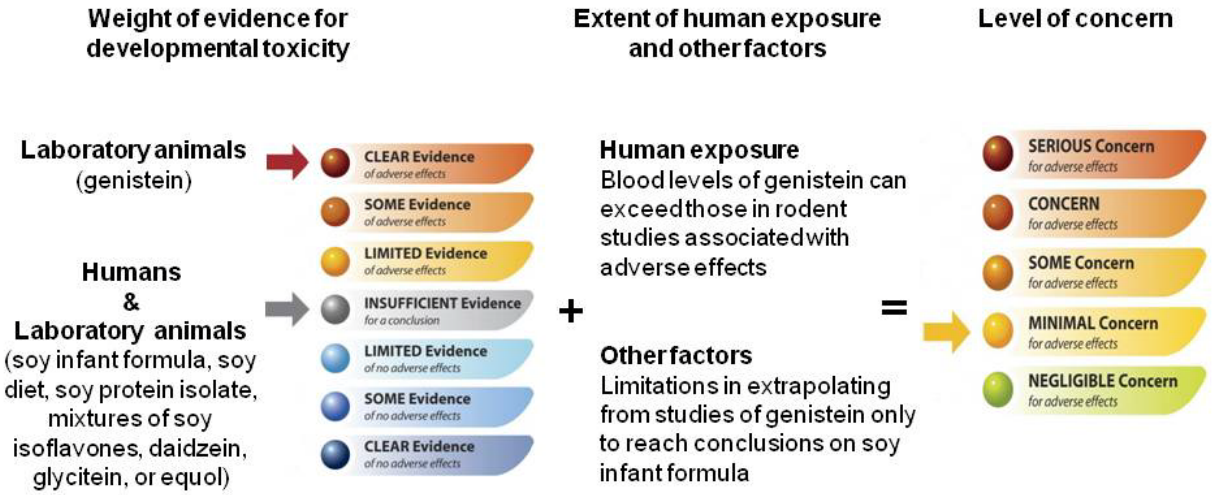
Basis for NTP Draft Conclusion of *Minimal Concern* for Adverse Effects on Development in Infants who Consume Soy Infant Formula



Charge

To determine whether the scientific information cited in the draft NTP Brief on Soy Infant Formula is technically correct, clearly stated, and supports the NTP's conclusions regarding the potential for soy infant formula to cause adverse developmental effects.

Action: NTP BSC will vote on whether the science cited in the draft NTP Brief on Soy Infant Formula supports the conclusion of *minimal concern* for adverse effects on development in infants who consume soy infant formula.